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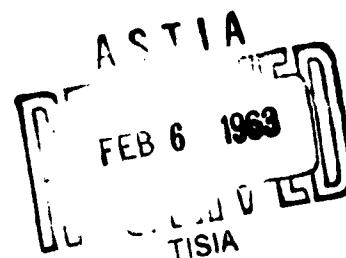
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## EXTRACTION APPARATUS FOR DETERMINATION OF TOTAL SERUM LIPIDS

TECHNICAL DOCUMENTARY REPORT NO. SAM-TDR-62-136

November 1962

USAF School of Aerospace Medicine  
Aerospace Medical Division (AFSC)  
Brooks Air Force Base, Texas



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CORRECTION

("Extraction Apparatus for Determination of Total Serum Lipids," by Dale A. Clark and Carrie B. Haven, SAM-TDR-62-136, Nov. 1962)

There is an error in the printing of line 19, column 1, page 3.  
It should read--

. . . equilibrated with  $1/5$  volume of water.

## **FOREWORD**

**This report was prepared by the following personnel of the Biokinetics Branch of the USAF School of Aerospace Medicine:**

**DALE A. CLARK, Ph.D.**

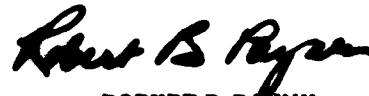
**CARRIE B. HAVEN, B.S.**

**The authors gratefully acknowledge the skillful assistance of Walter O. Baentsch, who fabricated the special glassware, and the help of Dr. Phelps Crump, who performed the statistical analyses.**

## ABSTRACT

Two designs of glassware useful in measurement of total lipids are described. One permits expulsion of the upper phase but increases requirements for solvent volumes. The other, a modified Erlenmeyer flask, facilitates withdrawal of the lower phase. Chilling the flask for 5 to 10 minutes in a refrigerator or deep freeze shortens the time required for separation of the two phases. Analyses of 7 samples by each of 3 methods in triplicate showed that the modified flask gives results that average 3 percent higher than results by method 2 of Sperry and Brand, but precision of both methods is the same. By using only 0.5 ml. of serum, the standard deviation of the results by either method is 2 percent.

This technical documentary report has been reviewed and is approved.



ROBERT B. PAYNE  
Colonel, USAF, MSC  
Chief, Operations Division



# EXTRACTION APPARATUS FOR DETERMINATION OF TOTAL SERUM LIPIDS

## 1. INTRODUCTION

The determination of total lipids in serum is frequently performed by using the method of Sperry and Brand (1), based on the work of Folch et al. (2). Details of the technics are elaborated in a review by Sperry (3). A recent report by Jacobs and Henry (4) provides evidence that this method gives excellent precision, minimal contamination of the lipid residue by nitrogenous residues or sodium chloride, and good phospholipid recovery.

One step in the determination is the purification of the chloroform-methanol serum extract by equilibration with water or a salt solution. This washing step is carried out with the chloroform-methanol extract in a beaker or glass cylinder instead of in a separatory funnel. The use of a beaker or a cylinder for separation of the chloroform and aqueous phases leads to inconvenience in withdrawing the upper phase completely. The large interface area between the phases makes complete removal of the upper phase difficult to accomplish. An apparatus in which the interface would be minimized during withdrawal of one phase would alleviate this problem. Two pieces of equipment designed for this purpose are described in this report.

## 2. EXPERIMENTAL

The flask separator (fig. 1) is an Erlenmeyer flask. To the bottom of the flask, there has been attached a capillary gooseneck sidearm, having a diameter of not less than 0.75 mm. nor greater than 1.25 mm. For the purification of 25 ml. or less of lipid extract, a 125 ml. Erlenmeyer flask is a convenient size

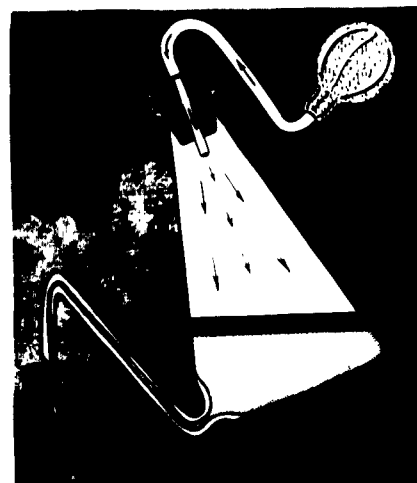


FIGURE 1

*Separator flask, showing expulsion of lower layer.*

to modify for use. The chloroform-methanol extract is introduced first into the separator flask and followed by the aqueous wash. This procedure prevents any of the aqueous phase from getting into the sidearm. The solutions are thoroughly mixed by swirling. Frequently, severe emulsions require long standing for complete separation. If desired, separation of the phases can be hastened greatly by chilling the flask with Dry Ice or by setting the flask in a deep freeze for 5 to 10 minutes.

After the phases have cleared, the lower one is expelled via the capillary sidearm. The operator uses a rubber bulb on one end of a length of small rubber tubing. The other end is attached to a glass tube that passes through a stopper which fits the mouth of the separation flask. The investigator exerts sufficient pressure to force the lower phase out the capillary sidearm. The flask is then tipped so that the outflow continues by gravity. As the



FIGURE 2

*Separator tube, showing removal of upper layer.*

last of the lower phase enters the capillary sidearm, the angle at which the flask is held is varied to control the rate of outflow. With a little practice, the operator can achieve precise control of the interface, stop it exactly at the tip of the sidearm, and effect a clean separation between the two phases. This result is best achieved by tipping the flask. If pressure is used to force the interface up the sidearm while the flask is held upright, droplets of the heavier phase frequently separate and remain behind in the ascending limb of the sidearm. Control of outflow by tipping the flask usually obviates this difficulty.

To effect quantitative recovery of the lipid extract, the flask must be rinsed with fresh chloroform-methanol solution. These wash solutions are introduced into the flask via the capillary sidearm by using gentle suction from the bulb. This procedure draws in any aqueous phase in the sidearm and rinses the capillary. After swirling the wash solution about the sides of the flask, separation of the two phases and expulsion of the lower one are carried out as before.

The design of this apparatus has several advantages. There are no stopcocks where solution might be lost. The flask provides a large volume for mixing solutions and a large

surface area for contact between upper and lower phases. During withdrawal of the lower phase, however, the final separation takes place in a capillary tube where the area of contact between the two phases is minute. Thus, clean separation of the two phases is possible. Since the lighter phase is not removed from the flask until all extractions and washings are complete, no loss of the original sample is possible except by errors such as contact of solutions with the stopper.

The apparatus illustrated in figure 2 is designed to facilitate removal of the upper layer. In use, the chloroform-methanol serum extract and aqueous wash solution are introduced through the sidearm. The short upper outlet is closed by thumb pressure, using a small piece of polyethylene to prevent contact of solvent with the thumb, and the unit is shaken the desired time. After the layers have separated (if necessary, by chilling as described above), the upper layer is expelled through the outlet by adding more chloroform-methanol solution through the sidearm. After the interface between the phases has been forced up into the capillary outlet, its position is accurately controlled by tilting the vessel.

It is possible to remove all of the upper phase with this apparatus, and, therefore, to dispense with the wash solution described by Sperry and Brand for their method 2 (1). However, the addition of chloroform-methanol solution, to displace the interface upward and expel the upper layer, necessarily increases the volume of the lower phase and lengthens the time required for subsequent evaporation of that layer. Furthermore, it is not feasible to wash either phase with fresh solution without transfer into another unit of separation. For these reasons, this design has not found routine use in our laboratory.

The separation flask illustrated in figure 1, however, has been used satisfactorily in this laboratory for several years, and is now in use in other laboratories. The design of this flask recommends itself to applications other than in determination of total lipids. In any extraction procedure using a separatory funnel, this

separator flask offers the possibility of effecting the separation without any loss of material in a stopcock. Furthermore, no bulky support is required for flasks sitting on a laboratory bench or chilling in a deep freezer.

The procedure for using this flask for total lipid determination is as follows: Prepare a filtered chloroform-methanol extract of serum as described by Sperry and Brand (1), but use only 0.5 ml. of serum (1 ml. of serum may, of course, be used, if available). Add 20 ml. of filtrate to the separator flask, and follow with 4 ml. of water. Mix by swirling for approximately one minute. Let stand or chill, as suggested above, until the layers separate. Then draw off the lower layer as described. Through the sidearm, draw in 2 ml. of fresh chloroform-methanol solution which has been previously equilibrated with 1.5 volume of water. Swirl the solution about to wash down the sides of the flask. After separation from the aqueous phase, draw off this lower phase and add to the first extract.

Use a stream of nitrogen to flush the air out of the flask containing the combined lipid extract and washings, and evaporate the solvent at temperatures not exceeding 40° C. on a rotating vacuum evaporator. Dissolve the residue in chloroform-methanol (2:1, v v) and filter through a fine porosity-sintered, glass filter into a weighed 5 ml. volumetric flask. Carry out the final evaporation and weighing as described by Sperry (3).

### 3. EVALUATION OF APPARATUS

The use of redistilled solvents was adopted after control experiments with the separator flask. A chloroform-methanol extract of 1 ml. of distilled water, instead of serum, was prepared, purified, and evaporated as usual. The weight of the final residue was calculated as apparent lipid concentration. The mean apparent lipid blank (3 experiments) using non-redistilled solvents was 51 mg. 100 ml. (range 38 to 61 mg. 100 ml.). By use of redistilled

TABLE I  
*Total lipids measured by using different glassware*

Sample	Cylinder	Separator flask	Separator tube
1	702 mg. 100 ml.	726 mg. 100 ml.	728 mg. 100 ml.
2	813	870	880
3	706	712	717
4	709	723	709
5	857	873	895
6	597	614	621
7	862	879	883
Mean	749.5	771.7	775.9
S.D.	15.5	16.0	21.0
S.D. as percent of total lipids	2.1	2.1	2.7
95% confidence limits of single determination, as percent of total lipids	4.5	4.5	5.8

Each recorded value is the mean of three determinations.

\*Standard deviation of distribution of measurements about individual sample means.

solvents, the mean apparent lipid blank (8 experiments) was 16 mg. 100 ml. (range 10 to 26 mg. 100 ml.). Efforts to reduce this blank to zero were unsuccessful, and it is therefore routinely subtracted from all total lipids determined by this method.

The cylinder (method 2 of Sperry and Brand (1)), the separator flask (fig. 1), and the separator tube (fig. 2) were compared by determining total lipids of seven different serum samples. By using each of the three pieces of apparatus, each sample was analyzed in triplicate, making a total of 9 determinations on each serum sample. The volume of serum used for each analysis was 0.5 ml. All solvents were redistilled before use. The chloroform-methanol (2:1) wash solution was first equilibrated with 1:5 volume of water.

The results of the triplicate analyses, together with the statistical analysis of the data, are summarized in table I. Method 2 of Sperry and Brand (1) gave a significantly lower mean value than either of the other 2 methods. However, all 3 methods were essentially equal in precision, as the similarity in standard deviation of the 3 methods shows. Although the standard deviation appears greater with the separator tube, this difference is not statistically significant. Since there was no interaction of sample with method, it was concluded that each method was consistent in what substances were being measured by that particular method.

The standard deviation of 2 percent of the total lipid is gratifyingly small, especially in view of the use of only 0.5 ml. of serum for the original extraction.

#### 4. DISCUSSION

It seems apparent that any of the 3 methods could be used to measure serum total lipids with reasonable assurance of consistent results. The meaning of the lower values obtained by the Sperry and Brand method is unknown. Although the difference is only 3 percent less than with the separator flask, this difference is real ( $P < .001$ ). It is possible that the flask method extracts some nonlipid materials. On the other hand, it is possible that a more nearly complete extraction of lipids is effected with the flask, because the additional solvent mixture used to rinse down the sides of the separation flask also extracts the aqueous phase again. In any case, the difference between the means is small, and both methods have equal precision. The 95 percent confidence limits of  $\pm 4.5$  percent obtained starting with 0.5 ml. serum in these experiments are reasonable in comparison with  $\pm 2.4$  percent observed by Jacobs and Henry (4), who started with 2 ml. serum. The use of the separator flask lends itself more conveniently to routine measurement of large numbers of samples because of the simplicity of withdrawing the lipid-containing phase.

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<p>USAF School of Aerospace Medicine, Brooks AF Base, Tex.</p> <p>SAM-TDR 62-136. EXTRACTION APPARATUS FOR DETERMINATION OF TOTAL SERUM LIPIDS. Nov. 62, 4 pp. incl. illus., table, 4 refs.</p> <p>Two designs of glassware useful in measurement of total lipids are described. One permits expulsion of the upper phase but increases requirements for solvent volumes. The other, a modified Erlenmeyer flask, facilitates withdrawal of the lower phase.</p>	<p>1. Lipids, determination of</p> <p>2. Apparatus, extraction</p> <p>3. Serum lipids</p> <p>I. AFSC Task 775301</p> <p>II. Clark, D. A., Haven, C. B.</p> <p>III. In ASTIA collection</p>	<p>USAF School of Aerospace Medicine, Brooks AF Base, Tex.</p> <p>SAM-TDR 62-136. EXTRACTION APPARATUS FOR DETERMINATION OF TOTAL SERUM LIPIDS. Nov. 62, 4 pp. incl. illus., table, 4 refs.</p> <p>Two designs of glassware useful in measurement of total lipids are described. One permits expulsion of the upper phase but increases requirements for solvent volumes. The other, a modified Erlenmeyer flask, facilitates withdrawal of the lower phase.</p>	<p>1. Lipids, determination of</p> <p>2. Apparatus, extraction</p> <p>3. Serum lipids</p> <p>I. AFSC Task 775301</p> <p>II. Clark, D. A., Haven, C. B.</p> <p>III. In ASTIA collection</p>
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